ASSOCIATION OF FLUCONAZOLE- TINIDAZOLE FOR THE TREATMENT OF VAGINAL INFECTIONS, ITS COMPOSITION, PREPARATION PROCESS AND USAGE

FIELD OF THE INVENTION

The present invention is related to the treatment of infectious diseases in the female reproductive system and more particularly to the use of an ASSOCIATION of compounds comprising fluconazole and tinidazole associated in doses lower to those usually administered therapeutically. This combination has proven to be highly effective and well tolerated.

BACKGROUND OF THE INVENTION

In medical practice vaginal infections are a common health hazard, since up to 95% of the patients go to the doctor due to vaginal flow. In primary health services, these infections affecting teenagers are found to be among the three topmost reasons for the visit, their incidence being much higher among the sexually active, although it has also been found among the girls who are not.

Normal vaginal secretions are inodorous, clear, viscous, with a Ph below 4.5, contain no necrophilia and do not flow during an examination with a speculum.

Among the factors favorable to vaginal infections we have poor genital-anal hygiene, a new sex partner or several of them, bathing in pools or tubs, pregnancy, diabetes, parasitic infections urinary or fecal incontinence, stress, congenital malformation, frequent use of antibiotics, hormones, contraceptive preparations to be used orally or topically and vaginal, immune system deficiency, tight clothes, nylon underwear, using non-hygienic vaginal elements used for the application of products and so forth.

The most frequent vaginal infections are shown in the table below.

Clinical Framework	Etiological Agent
Scarce whitish flow, pH < 4,5	*Candida albicans*
Vulva itch and/or irritation, erythema, profuse yellow flow, pH>5	*Trichomonas vaginallis*
Vulva itch, malodorous white-grayish flow, pH > 4.5	Gadnerella *vaginallis* (Haemophilus

	vaginallis)
A smell of amines	Anaerobes (peptostreptococcus bacteria, porphyromons) after the addition of potassium hydroxide Mobiluncus spp
10%* presence of guide cells	*Mycoplasma hominis* (Amsel's Criteria)
Abnormal flow, post-coital bleeding	Chlamidia trachomatis

Vaginal infection or vaginal flow syndrome is an infectious process characterized by one or more of the following symptoms: flow, vulva itch, burning, irritation, dysuria, dyspareunia and vaginal malodor; a microorganism is often to be found during vaginal infection, which originates mixed vaginal infections.

Vulvovaginitis, vulvitis and vaginitis are terms usually related to a swelling of the vagina or vulva, frequently caused by fungi, bacteria and parasites. By vulvovaginitis we understand the anomalous and irritating flow of secretion, whether malodorous or not, which produces local discomfort (itching or burning sensation)

and which can be accompanied by dysuria and/or dyspareunia. Vulvovaginitis is the most frequent gynecological problem leading to a first visit to the doctor. 90% of females showing symptoms suffer from a bacteria sort of infection (mostly *Gardnerellas*), candidiasis or *Trichomonas*. The remaining 10% show a different set of symptoms: ETS, vaginal atrophy, allergies and chemically induced irritation. Candidiasic vulvovaginitis (VVC) is the most common cause of vaginitis in Europe. About 85-90% of the cases are due to *Candida Albicans*. The initial treatment makes use of topical agents.

Initial treatment make use of topical agents such as creams, vaginal tablets, and ovules for periods from 7 to 10 days, with the ovules and creams a recovery rate of no more than 75% percent is achieved. Due to the high level of discomfort for the patient and the length of the treatment, other options for the treatment of vaginal infections have been sought, such as the use of systemic treatments and even the shortening of the period of the treatment itself. Some treatments may include the use of clotrimazole, miconazole, fenticonazole and nistatine. In the case of severe infections, recurrent ones or intolerance to vaginal application, the use of 400 mg a day of ketoconazole for 5 days or 200mg of itraconazole for 3

days or 400mg 400 mg one day or 150 mg of fluconazole for one day is recommended.

In the case of highly severe or chronic infections (4 or more episodes a year) an oral treatment is used and, to avoid a recurrence, 100 mg of ketoconazole a day is used during 6 months, a vaginal ovule of cotrimazol a month in the pre-menstrual stage during 6 months, or 200 mg of itraconazole taken orally for 3 days during 6 cycles.

Bacteria induced vaginitis represents an alteration of the vaginal flora characterized by a decrease in the concentration of hydrogen peroxide produced by the lactobacilli and an increase in the prevalence of *Garnerella vaginalis*, and negative gram anaerobes which bring about the onset of malodorous flow without showing any signs of vulvo vaginal swelling. In bacteria induced vaginitis most of the lactobacilli disappear, vaginal pH increases and a pathogenic proliferation of other anaerobes bacteria is found. There are four bacteria associated to bacteria induced vaginitis: *Gardenerella vaginalis*, a facultative, fermentative anaerobes present in 40% of the average female, and the most commonly associated to this pathological condition (95%); *Mobiluncus*, *Mycoplasma hominis* as examples of negative gram anaerobes, and *Peptostreptococcus*. By oral via the preferred treatment is metronidazole: 50 mg taken orally, every 12 hours for 7

days. As alternative paths 2 g of metronidazole in an only dose or 300 mg of clindamycin taken orally twice a day for a week are used.

Vulvovaginitis due to *Trichomona* is a process caused by mobile protozoa, flagellate and anaerobic called *Trichomona vaginalis* and is acquired due to sexual intercourse. It is one of the main causes of vaginal infections. Fifty per cent of the patients (both male and female) are non symptomatic at the moment of the diagnoses. A third of them will develop the symptoms in the following 6 months if not treated. The most recommended pattern for the treatment is to take 2 grams of metronidazole in an only dose, whether male or female. By means of this therapeutic regime, and if also the sexual partner is involved, up to 85% of efficacy can be reached. In the event of therapeutic failure, an alternative regime of 500 mg metronidazole every 12 hours for 7 days can be prescribed. Should the infections be resistant, either tinidazole or furazolidone will be used.

Taking into account that in the same woman several clinical variations may coexist and that the presence of a particular form of etiological agent cannot be fully determined by a gynecologic exam, the treatment should be approached as syndromes; concentrating on the most common infections associated to the vaginal flow

syndrome: trichomoniasis, candidiasis and bacterial vaginosis as in those cases the infection is mixed.

The following table shows a resume of the most commonly prescribed treatments:

Table 1

Candidiasis	**Trichomoniasis**
-Isoconazole, 1%, vaginal cream, 7 to 14 days.	-Metronidazole, 250 mg, orally, 3xday, 7 days
-Miconazole, cream or ovules, 7 days	-Metronidazole, 2.0 g, orally, one dose
-Tioconazole 6.5%, topical, one dose	-Tinidazole, 2.0 g oral, one dose
-Terconazole, cream at 0.8%, 7 days	-Secnidazole 2.0g, oral, one dose
-Fluconazole 150g oral, one day	-Miconazole + Tinidazole associated, vaginal cream, 7 days
Itraconazole 400mg-1 day or 200mg, 3 days	
Ketoconazole 200mg, 2 tablets, oral, 5 days	
Gardnerella	**Neisseria/Clamydia**
-Metronidazole, 500 mg. oral, twice a day, 7 days	-Ceftriaxone 250mg,IM one dose

-Tinidazole 2.0g, oral, one dose	-Azithromycin 1.0 oral, one dose
Clindamycin 30mg oral, 2 a day, 7 days	-Doxociline 100mg oral, 2 a day, 7 days
Clindamycin 2% vaginal cream 3 to 7 days	-Ofloxacin 300mg oral 2 a day, 7 days
	-Eritromicin stearate 500mg oral 4 times a day, 7 days

In some countries, changes in the infection patterns have been reported as a result of changes in sexual behavior. *Chlamidia*, genital herpes and papilloma are at present more frequent than gonorrhea and syphilis. Just in the United States, from 8 to 10 million new cases of *Chlamidia* are reported every year. Strains of *Neisseria gonorrhoeae* resistant to both penicillin and tetracycline have become widespread. Aside from that, the *Trichomonas vaginalis* resistance to metronidazole has varied.

In the state of the art there are several published works where reducing the length of the treatment is being sought. A case in point is the patent MX 188,752, wherein a therapeutic method for the treatment of vaginal infections is described and which includes using a combination of itraconazole-secnidazole. The treatment described in the patent mentioned above is quite lengthy

and demands taking 12 doses during the treatment for 3 days, which makes the fulfillment of the treatment by the patient questionable. Recovery is estimated at 77.77%.

In the patent application No. 02/07641, a codependent application to the present one, a fluconazole- and tinidazole pharmaceutical combination is described, its main feature being that the doses used are 150 mg fluconazole and 2g tinidazole for the treatment of infectious diseases in the female reproductive system.

In the present invention, quite surprisingly, the association of fluconazole and tinidazole in lower doses to those known, and administered in only one day of treatment in one or two events, was discovered to improve in all aspects over the known treatments for mixed infections in the human reproductive system- a feature that represents an edge over other lengthier treatments as it ensures that the patient will follow the treatment much more closely.

Contrary to expectations, diminishing significantly the dose did not affect the effectiveness of the fluconazole-tinidazole association, which held, therefore abating the secondary effects. of these substances while keeping their effectiveness.

Another advantage the treatment has is that it is very useful for the medical personnel and communities that,

due to various factors, can only access to a clinical diagnostic.

A further advantage is the higher possibility of therapeutic success, allowing for a quick lessening of the symptoms, with a good level of tolerance and acceptance from the part of the patient.

Objectives of the Invention

One of the objects of the present invention is to provide effective relief from mixed infections in the human reproductive apparatus by means of the use of a fluconazole-tinidazole association in lower doses than those already described in the state-of-the-art.

Another object is to cure this kind of infection quickly and efficaciously.

Still another objective of the present invention is to have the patients' total acceptance since it is an only dose.

A further objective is to avoid the patient abandoning the treatment as it takes only one day.

An additional aim is to prevent the development of complications in the upper genital tract.

And, still a further aim is to provide a compound using the fluconazole-tinidazole association for the

treatment of infectious diseases in the human reproductive system, which will improve over the traditionally known treatments.

Detailed description of the invention

The present invention is related to the treatment of infectious diseases in the human reproductive system, masculine and feminine by means of the use of a composition which consists of an association of two chemical substances: fluconazole and tinidazole, used in lower doses to those known. Such a combination has proven to be highly efficacious.

The composition of the present invention is administered in one and up to two events, which represents a really important advantage over longer treatments since the patients' acceptance is higher. This, in turn, ensures the patients' following the treatment to its completion.

Fluconazole is a triasolic-derived substance with a wide antimycotic effect; its fusion point is between 138 and 140°C. Its molecular polarity allows for a systemic action both when it is taken orally or parenterally.

Insofar as the pharmacokinetics of fluconazole, this substance is well absorbed, showing total bioavailability of over 90% and, in general, is not

affected by medication that modifies the gastrointestinal pH. Neither is its absorbance affected by food ingestion. Furthermore, it distributes itself all over the organism. Due to a low union with plasmatic proteins, the vagina has shown a very smooth distribution of this medication, as the relationship between plasma and the vaginal tissue after oral administration is of 0.94 to 1.1, (which indicates that the concentrations are practically similar) while in vaginal flow, the relationship is 0.5 to 1.0 in respect to plasma and it has proven to be highly effective in the treatment of vaginal candidiasis. In an epidemiological survey, conducted in 1,017 patients suffering from vaginal candidiasis and who were given one dose of 150mg, fluconazole was well received and effective in 91% of the patients. The appearance of adverse side effects was less than 1%.

Its widespread distribution both in the body tissues and liquids, as well as its 25 to 30 hours life span may account for its effectiveness, both in the short and in the long run. Its prolonged elimination life also contributes to its efficacy.

Tinidazole is a 5-nitroimidazole derivate with selective activity against anaerobes bacteria and protozoan. Tinidazole is totally absorbed orally and spreads throughout the organism. It has a low attachment

to proteins, it being 12%. The active mechanism is similar to that of nitroimidazole derivates that produce a bactericidal effect by means of forming toxic metabolites, which brings about the rupture of DNA. The same as other antibiotics that inhibit protein synthesis or affect nucleic acids, nitroimidazole has a post antibiotic effect. Clinical research has shown that tinidazole is effective in the treatment of respiratory infections, intra-abdominal sepsis, amoeba infection, giardasis and gynecological infections produced by *Trichomona vaginallis*. In the treatment of Bacterial Vaginosis, to which *Gardnerella vaginallis* is often associated, using an only doses of 2g tinidazole, yielded a curative rate of 92%, and other researchers using the same amount in a two-day scheme have reported rates of 51%.

Secnidazole is endowed with an anti-parasitical activity and acts against *Entamoeba histolytica, Giardia lamblia, Trichomona vaginallis* and *Gardnerella vaginallis*. After the oral administration of one 2g dose of secnidazole, the highest seric rates are obtained on the third hour. Average plasmatic life is of about 25 hours. Elimination, essentially urinary, is slow (50% of the doses taken is excreted in 120 hours).

The present composition which comprises an association of two chemical products, as the active

principle, in lower doses to the ones commonly known for the treatment of vaginal infections by its activity spectrum, allows for the treatment both of the vaginitis caused by *Candida sp* and *Trichomona vaginallis*, as the one caused by *Gardnerella* and anaerobes bacteria.

Due to the proportions used in the new fluconazole-tinidazole composition, a similar or higher inhibition of the microorganisms which cause mixed vaginal infections as regards the conventional treatments and the dose usually known are obtained.

In the present invention, the composition comprises a fluconazole-tinidazole combination. The weight relationship is from 50 to less than 150 mg of fluconazole and from 1000 to less than 2000 mg of tinidazole. In a preferred embodiment the weight proportion is 112.5 fluconazole to 1500 mg tinidazole. These last values mean diminishing fluconazol from 150 mg to 112,5 mg, that is, 25% less and from 2000 mg to 1500 mg, that is, a 25% lower dose than that reported in the published works.

A skilled in the art could expect nothing but a lowering of the therapeutic effect of the medication when the dose is lowered in the amount mentioned, which, as will be shown, does not happen.

The pharmaceutical composition for the treatment of vaginal infections in the present invention, is better

taken orally in a wide variety of pharmacy presentations such as Capsules, tablets, pills, effervescent tablets and sublingual tablets, not being limitative in any of them.

In a preferred, but not limitative fashion, the tablets used for the treatment of mixed vaginal infections comprise the fluconazole-tinidazole association in a dose lower to those therapeutically known to date and at least one pharmaceutically acceptable vehicle. Secnidazole is considered to be an alternative ingredient to tinidazole.

Among the pharmaceutically acceptable vehicles we can count, not limiting ourselves to silicon oxide, varieties of glycolate, crospovidone, sodium PVP lauril sulfate, magnesium stearate, isopropyl alcohol.

As regards the preparation of the capsules or tablets for the treatment of mixed vaginal infections, they require an unconventional manufacturing process, given the unusual fluconazole-tinidazole proportion. In order to obtain an excellent uniformity in the product, the process must be controlled. The integration of the fluconazole-tinidazole substances is carried out by making an agglutinating solution, including the chemical product in a lower proportion and that subsequently is used as the granulating solution, wherein the substance with the lower proportion is fluconazole-tinidazole; tinidazole as well as the other components are mixed in a fluid bed to which the

agglutinating solution is added to obtain the granular form. The steps subsequent to this one, are the ones commonly used in the making of tablets: drying, grinding and compressing.

The following examples are meant to illustrate this invention, the same not being limitative.

Examples of composition

Example 1

A pharmaceutical composition including a fluconazole-ticonazole association in the form of tablets is prepared according to the following composition:

Ingredient	**Quantity**
Tinidazole	500 mg
Fluconazole	37.5 mg
Microcrystalline Cellulose 101	60.50 mg
Sodium glycolate of starch	6.50 mg
Crospovidone	16.25 mg
Lauril sodium sulfate	6.50 mg
Polyvinylpirrolidone K-30	19.5 mg
Magnesium stearate	3.25 mg
White Opadry YS 7322	10.5 mg
Total	660.5 mg

Example 2

A pharmaceutical composition including a fluconazole-ticonazole association in the form of tablets is prepared according to the following composition:

Ingredient	**Quantity**
Tinidazole	750 mg
Fluconazole	56.25 mg
Microcrystalline Cellulose 101	90.75 mg
Sodium glycolate of starch	9.75 mg
Crospovidone	24.37 mg
Lauril sodium sulfate	9.75 mg
Polyvinylpyrrolidone K-30	29.25 mg
Magnesium stearate	4.87 mg
White Opadry YS 7322	15.75 mg
Total	1321 mg

Example 3

A pharmaceutical composition including a fluconazole-ticonazole association in the form of tablet is prepared according to the following composition:

Ingredient	Quantity
Tinidazole	1500 mg
Fluconazole	112.5 mg
Microcrytalline Cellulose 101	181.5 mg
Sodium glycolate of starch	18.15 mg
Crospovidone	48.75 mg
Lauril sodium sulfate	19.5 mg
Polyvinylpyrrolidone K-30	58.5 mg
Magnesium stearate	9.75 mg
White Opadry YS 7322	31.5 mg
Total	1981.5 mg

Example 4

A pharmaceutical composition including a fluconazole-Secnidazole association in the form of tablets is prepared according to the following composition:

Ingredient	Quantity
Secnidazole	500 mg
Fluconazole	37.5 mg
Microcrytalline Cellulose 101	60.50 mg
Sodium glycolate of starch	6.50 mg
Crospovidone	16.25 mg
Lauril sodium sulfate	6.50 mg

Polyvinylpyrrolidone K-30	19.5 mg
Magnesium stearate	3.25 mg
White Opadry YS 7322	10.5 mg
Total	660.5 mg

Pharmacological examples

A longitudinal study, comparative with a simple random assignation was conducted. The study included 42 females over 18 years old, not pregnant, who showed signs of vaginal infections.

The patients were divided into two groups; Group 1 was given one 150mg dose of fluconazole and a 2 g tinidazole (standard dose), which is that reported in the co-pendant application of the present application. Group 2 was given a 112.5 mg fluconazole 1500 mg tinidazole association; it was the second group, representing the preferred dose in the present application, an evidently lower dose than the one of the state of the art, yielded surprising results. The medication was given to both groups on two events during one day.

All the patients submitted to a gynecological exploration in order to determine the characteristics of vaginal discharge and the accompanying symptoms were recorded. A pre and post treatment vaginal culture was

conducted. The patients were asked to refrain from sexual intercourse in the interval between the taking of the vaginal cultures.

The initial symptoms evaluated were: odor, itching, vulva irritation, dyspareunia and vaginal secretion, which decreased significantly after the treatment.

The results of the initial culture are shown in the following table:

Infection	No of cases
Gardnerella Vaginalis	29
Gardnerella and *Actinomyces*	1
Gardnerella and *Candida*	3
Gardnerella and *Micrococcus*	1
Bacterial Vaginosis	2
B.Vaginosis and *yeast*	1
B. Vaginosis and *Proteus*	1
B. Vaginosis and *micrococcus*	1
B. Vaginosis and *E. Coli*	1
Trichomonas Vaginalis	1
Trichomonas V.+Gardnerella+E. Coli	1
Total number of cases	2

The response to the treatment was as follows:

In the group which was given the standard dose, microbiological eradication was of 82%, while in the group that took the lower dose (i.e. the dose of present application) eradication was of 80%, which showed that, statistically, there is no significant difference between both groups.

Table 2- Comparing efficacy of treatments:

Eradicating germs sensitive to Tx.	Treatments			P. value
	Inventive dose	Standard dose	Total	
Yes	16 (80%)	18 (82%)	34	0.8690
No	4 (20%)	4 (18%)	8	
Total	20 (100%)	22 (100%)	42	

As regards adverse effects, only one patient reported to having suffered dizziness and 3 complained from episgastralgia, all of which were temporary.

The fuconazole-tinidazole combination showed to be effective in the treatment of the most commonly found germs in clinical practice of infections in the reproductive system, both in the standard and in the low dose, the adverse effects not having been regarded as

important. This is because the minimum inhibiting concentrations of this composition makes it possible to reach an eradication percentage similar to the eradication percentages already usually obtained by 150 mg fluconazole and 2.0 g tinidazole

With the present invention it was unexpectedly found that, in doses lower to those already known of the fluconazole-tinidazole chemical compounds, the same therapeutic effects were achieved but with fewer adverse effects.

It is believed that Applicant's invention includes many other embodiments which are not herein specifically described accordingly this disclosure should not be read as being limited to the foregoing examples or preferred embodiments.